PROCEDURE FOR LAB ANIMAL 2 PLATE ELISA ASSAYS

Research Reagents for the Detection of Antibodies in animal sera by ELISA

Store at 2-8°C
For Research Use Only

REAGENTS:

Sufficient reagents are supplied to run 96 tests.

NOTE: Kit plate and conjugate lots are matched and must be used as a set. All reagents are ready-to-use EXCEPT the wash concentrate is supplied as a 20X concentrate.

ELISA Strips	2 strip holders containing 6 positive viral antigen coated strips and 6 negative antigen coated strips each (alternating + and - antigen strips)
Sample Diluent	2 bottles, 30 ml each
Peroxidase Conjugate	2 vials, 12 ml each
ABTS Peroxidase Substrate	2 vials, 12 ml each
Stop Solution	1 vial, 10 ml
Wash Concentrate (20X)	2 vials, 60 ml each
Positive Control Serum	1 vial of 1ml
Negative Control Serum	1 vial of 1ml

STORAGE AND STABILITY INSTRUCTIONS

1. Store all reagents at 2 - 8°C when not in use. The expiration date printed on the box label indicates the limit of stability of the product.
2. The foil packs containing the ELISA strips should be allowed to warm to room temperature (20 - 25°C) before opening to prevent condensation. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained.

INSTRUCTIONS FOR USE

Reagent Preparation Prepare the following reagents and samples before beginning the assay procedure. All reagents and samples should be at room temperature (20 - 25 °C) prior to beginning the assay and may remain at room temperature during testing. Return reagents to 2 – 8°C immediately after use.

Sample Diluent – 2 vials (30 ml/vial)
Contains normal goat and bovine serum in phosphate buffered saline and Proclin as a preservative. The Sample Diluent is stable for a minimum of 1 year when stored at 2 - 8°C.

POSITIVE Control Serum - 1 vial (1 ml)
This vial contains positive control serum ready to use at the dilution to be used in the test. No further dilutions are required. The Control Serum is stable for a minimum of one year when stored at 2-8°C.

NEGATIVE Control Serum - 1 vial (1 ml)
This vial contains negative control serum ready to use at the dilution to be used in the test. No further dilutions are required. The Control Serum is stable for a minimum of one year when stored at 2 - 8°C.

Wash Concentrate (20X) - 2 vials (60 ml/vial)
Contains tris buffer with surfactant. Check the Wash Concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37◦C until crystals dissolve. Wash Solution is stable for 3 weeks from date of preparation if stored at 2-8°C. Therefore, dilute Wash Concentrate as needed.
Dilute the Wash Concentrate 1:20 with deionized or distilled water in a clean glass or plastic screw cap container (for example: add 50 ml of wash concentrate to 950 ml of water). Mix gently by inverting several times to avoid excessive foaming. When using an automated plate washer, to ensure sufficient volume, 1 x 60 ml of Wash Concentrate has been provided to allow for excess Wash Solution to prime the plate washer (2400 ml total Wash Solution after dilution).

Peroxidase Conjugate - 2 vials (12 ml/vial)
The Conjugate is provided ready to use. One vial provides enough Conjugate for 12 strips (1 plate). If more than 1 vial is required, pool the contents of both vials in a clean glass or plastic screw cap container and mix gently by inverting several times to avoid excessive foaming. Opened Conjugate is stable for 30 days. Label and date the vial and store at 2-8°C.

ABTS Peroxidase Substrate - 2 vials (12 ml/vial)
Each vial contains 12 mls of 2,2' Azino-di[3-ethyl-benzthiazoline-sulfonate] solution. The substrate is ready for use. ABTS is stable for a minimum of one year at 2-8°C.

Stop Solution - 1 vial (10 ml)
Contains 1.25% sodium fluoride. CAUTION: Avoid contact with eyes and skin. If contact is made, wash area with copious amounts of water and seek immediate medical attention.

SPECIMEN COLLECTION AND PREPARATION

A. Obtain blood and allow clot to form. Insoluble materials should be removed by centrifugation. Remove the serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be aliquotted and frozen. Avoid repeated freezing/thawing of samples. Samples should not contain sodium azide.

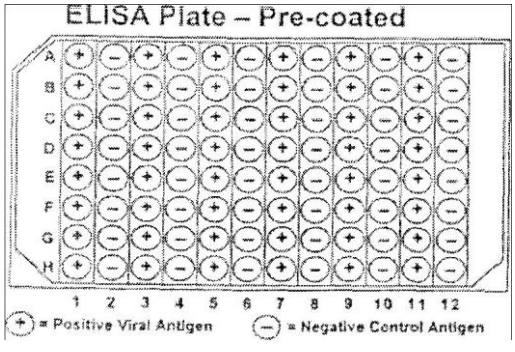
B. Dilute the serum 1:50 in Sample Diluent. For example: add 5 ul of serum sample to 245 ul of 1X Sample Diluent. If not assayed immediately, diluted samples should be stored at -20°C or below.

Procedural Notes

1. Review the complete instructions before performing the test.
2. Strips of the ELISA plate are removable. Remove unused strips and store as described in "Storage and Stability Instructions." Before testing begins, the user should inspect the ELISA strip holders and ensure that all strips are secure. A white stabilizer residue is normally observed in the bottom of unused wells.

Strip holders should be handled with care to ensure that no strip is dislodged during testing. It is recommended that each strip be numbered with a laboratory marker prior to use. Additionally, since the strips are pre-coated with positive viral antigen and negative control antigen (alternating 6 positive and 6 negative antigen-coated strips/strip holder), it is recommended that each strip be labeled with a "+" or "-" to indicate type of antigen coat in each well.

NOTE: Assembled strip holders always start with a positive antigen (+) coated strip. Subsequent strips alternate between negative control (-) antigen and positive (+) antigen, so that strips 1,3,5,7,9 and 11 are pre-coated with positive (+) antigen and strips 2,4,6,8, 10 and 12 are pre-coated with negative (-) control antigen. A schematic representation of this is below.



Avoid touching bottom surfaces of wells, as this may affect readings. ELISA strips must be used only once.
Strip holders may be used again.

3. Dispose of all used materials as biohazardous waste.
4. A new pipette tip must be used for each sample, never touching pipette tip to the bottom of the well. If plastic troughs are used, ensure that they have a dedicated purpose (do not use the same trough for Peroxidase Conjugate and ABTS Peroxidase Substrate).

Wash Procedure

Proper washing and aspiration of the stripwells is required to obtain accurate, reliable results. Wells should be washed 5 times after both Sample incubation and after Conjugate incubation.

1. Aspirate wells into a waste flask.
2. Fill each well with Wash Solution.
3. Aspirate wells.
4. Repeat steps 2 and 3 for an additional 4 cycles (Total, 5 washes).
5. Invert plate and tap firmly on absorbent paper to remove excess liquid. Take care not to dislodge strips.

Test Procedure

All samples and Controls should be tested on both the positive viral antigen and the negative control antigen wells. Use the enclosed record sheet to identify the location of each serum and type of strip (+ or - antigen) used in the test.

1. Make a 1:50 dilution of the test serum in 1X Sample Diluent in a small dilution tube and mix well. EXAMPLE: Add 5 ul of serum to 245 ul of 1X Sample Diluent.
2. Fit the strip holder with the required number of pre-coated Positive Viral Antigen and Negative Control Antigen strips. Mark the appropriate strips with a (+) or (-). Allow one well to be used for the Negative Control Sera and one well for the Positive Control Sera.
3. Pipette 100 ul each of the diluted serum sample, the Negative Control and positive Control into the appropriate (+) and (-) marked wells.
4. Cover the wells and incubate at 37°C for 45± 1 minutes.
5. After incubation, wash each well five (5) times with 1X Wash Solution (refer to Wash Procedure).
6. Pipette 100 ul of liquid, ready-to-use Peroxidase Conjugate into each test well. Cover the wells and incubate at 37°C for 45± 1 minutes.
7. After incubation, wash each well five (5) times with 1 X Wash Solution (refer to Wash Procedure).
8. Pipette 100 ul of liquid, ready-to-use ABTS Peroxidase Substrate into each test well.
9. Incubate the plate at room temperature (20 - 25°C) for 30 minutes. Do not cover the plate.
10. Blank the micro reader on air and read the absorbance of the colorimetric reaction in each well at 405nm.
11. If the plate is not read immediately, pipette 25 ul of Stop Solution into each test well. Read the plate at 405 nm within 15 minutes.

INTERPRETATION OF RESULTS

1. It is recommended that each laboratory establish their own criteria for performance of these Research Reagents.
2. In our quality control testing, we use the following criteria:
   a. The Negative Control Serum, after subtracting the absorbance in the negative control antigen well, should produce a net absorbance on the Positive Viral Antigen of ≤ 0.250 at 405 nm.
   b. The Positive Control Serum, after subtracting the absorbance in the negative control antigen well, should produce a net absorbance on the Positive Viral Antigen of ≥ 0.600 at 405 nm.
   c. A sample may be considered positive by the following criteria: Determine the difference (Δ) between the sample absorbance at 405 nm on the Positive Viral Antigen well and the absorbance at 405 nm on the Negative Control Antigen well. **This difference (Δ) should be greater than or equal to 0.300 for a sample to be considered positive.**

Example # 1: Positive Sample
Given a sample absorbance of **1.101** at 405 nm on the Positive Viral Antigen well and a sample absorbance of **0.190** at 405 nm on the Negative Control Antigen well.
The difference (Δ) between the above absorbances is **0.911.**
This difference is greater than or equal to **0.300**. This sample is considered Positive.

Example # 2: Negative Sample
Given a sample absorbance of **0.347** at 405 nm on the Positive Viral Antigen well and a sample absorbance of **0.319** at 405 nm on the Negative Control Antigen well.
The difference (Δ) between the above absorbances is **0.028.**
This difference is less than 0.300. This sample is considered negative.

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